

Original Research Article

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Phytochemical Screening and Antibacterial Activity of *Erythrina variegata* L. (Leaf Extract)

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ABSTRACT

Various extracts on the basis of polarity of solvent of *Erythrina variegata* L. (family-fabaceae) leaves such as hexane, chloroform, dichloromethane, ethyl acetate, methanol and aqueous were evaluated for their phytochemicals and antibacterial activity against selected five bacterial culture viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* by disc diffusion method. The activities of the samples were compared with that of standard antibiotics e.g. Amikacin. Phytochemical screening of *Erythrina variegata* leaf extract in five different solvents showed the presence of important phyto-constituents like phenols, alkaloids, flavonoids, tannins and saponins. In antibacterial assay, all the organisms respond to the plant extract but inhibitory zone developed. Among the selected solvent used in Soxhlet apparatus extract were hexane, chloroform, dichloromethane, ethyl acetate, methanol and aqueous, all developed inhibition zone against bacteria; this means selected plant has antibacterial property. The chloroform extract showed a higher activity than other extracts. The methanolic leaf extract of *E. variegata* showed higher activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* followed by *Klebsiella pneumoniae*, *Proteus vulgaris* and *Escherichia coli*. The methanolic leaf extract of *E. variegata* showed maximum zone of inhibition against *Staphylococcus aureus*. The ethyl acetate extract of *E. variegata* exhibited high activity against *E. coli* and *Staphylococcus aureus* followed by methanol, chloroform, hexane and aqueous extracts. The results revealed that the antimicrobial activity exhibited by *E. variegata* chloroform and aqueous extract noted to be most effective than other solvents.

Keywords

Antimicrobial activity, Antibiotics, *Erythrina variegata* L. (Leaves), Phytochemical screening.

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Introduction

Protective or disease preventive properties of phytochemicals are widely known and recognized. They are non-nutritive plant based chemical which are supposed to be non-essential for the human body. However, researches have proved that many phytochemicals can be highly useful in protecting humans against diseases. Even today, over 80% of the world population, especially in the developing world practices

traditional medicine prepared from plant extract for their health and wellness (Viji, 2010). Natural plants are significantly important for their role in medicine and plant metabolites became major player in recent drug discovery and formulation. They are important source of noble drugs compounds which results in plant derived medicines (Cardero *et al.*, 2012). It is well known that secondary metabolites are a source of

biologically active natural products with various functions, including antibacterial, antifungal, antiviral, antineoplastic and anticancer activities, acting also as inhibitors and plant growth promoters (AL-Zereini, 2006). The major advantage offered by the use of these metabolites as useful templates, is that they are per se highly active and selective. They can also be used within the structure of its active sites, in order to develop new substances with predetermined properties (Gonzalez and Silva, 2001). In addition, chemical research related to the isolation, biosynthesis and structural elucidation of new natural compounds has contributed to pharmaceutical progress, for example, in treatment of diseases, pests and the development of new chemicals (AL-Zereini, 2006).

Plant of *Erythrina variegata* (Division: Magnoliophyta – Flowering plants, Class: Magnoliopsida – Dicotyledons, Family: Fabaceae (Legume family), Subfamily: Papilionoideae, Genus: *Erythrina* L. – Coral Tree, Species: *E. variegata* L.) is universally recognized for their high biomass productivity and utilization. This plant is reported to be a highly popular cultivated plant in India having deciduous tree shaped, upto 13 m tall; unarmed; bark yellowish or greenish grey, smooth; wood white; young branches pubescent, prickled, which fall when a few years old. Leaves 3-foliolate, rachis 20-30 cm; petiole 15-20 cm; leaflets thin coriaceous, ovate-rhomboid, 5-15 x 5.5-17 cm, glabrous, apex acuminate, margin entire, base truncate, obtuse; secondary nerves 5-6 pairs, petiolule to 8 mm. Flowers scarlet, 2.5 cm across in 5-15 cm long, dense racemes. Pod torulose, 20-35 x 2.5-3 cm, deep brown, stipulate; seeds 7-12, 1.5-1.8 cm long, dark purple (Pullaiah and Sandhaya Rani, 1999). Different parts of *E. variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antilepileptic. In some experiments, it has

potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds (Warrier *et al.*, 1994, Gupta *et al.*, 2002., Ghosal *et al.*, 1972). We therefore undertook this study to evaluate the phytochemical constituents and antibacterial potential of the various solvent extracts, from the leaves of *E. variegata* (Muthukrishnana, 2014). However, few scientific and systematic studies have been done on phytochemical screening and isolation of secondary metabolites in *E. variegata* for their antibacterial efficacy. Considering international interest in herbal medicine research on this underutilized plant as a source of bioactive chemicals was carried out with the objective of evaluating its potential antibiotic activities to make a diagnosis of the chemistry related to the plant extract of *E. variegata* in order to explore new and noble bioactive compounds (Srinivasan, *et al.*, 2001).

Materials and Methods

Collection of plant sample

The fresh leaves of *E. variegata* were collected from Agamkuan near RMRIMS, Patna randomly.

Preparation of leaf extracts

The leaves were washed thoroughly with tap water and in distilled water and then shaded dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 100gm of powdered plant material was uniformly packed into a thimble made up of muslin cloth and extracted with 500ml of different solvents separately in Soxhlet apparatus. Solvents used were hexane, chloroform, dichloromethane, ethyl acetate, methanol and aqueous on the basis of their polarity. The process of

extraction continues till the solvent in tube of an extractor become colorless. The extracts were filtered through muslin cloth or Whatman No.1 filter paper and collected in brown bottle separately. After that the different extract solvent was evaporated by rota vapour separately, where the temperature of water bath should be at 40⁰C, which gave rise to a solid mass of the extract. The solid mass was refrigerated for further use.

Phytochemical analysis

The qualitative analysis of tannins, phenols, glycosides, alkaloids, steroids and flavonoids were analyzed by standard method (Harborne, 1973).

Selected test microorganisms

Extracts were tested against pathogenic microbes, including the bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*.

Antibacterial assay

Antibacterial activity of plant extracts was carried using agar disc diffusion method with some minor modifications. (200µl bacterial cultures were spread in nutrient agar culture plate by glass rod spreader. Disc of 6mm of plant different solvent extract along with positive control and negative control were diffused in the Petri plate and after incubation, zone of inhibition were read by mm scale (Murray, 2009).

Results and Discussion

Phytochemical screening of *Erythrina variegata*

Presence and absence of primary phytochemicals viz., alkaloids, flavonoids, glycosides, steroids, phenols, tannins,

saponins and resins was confirmed in the laboratory tests.

Some plant extracts and phytochemicals are known to have antibacterial properties, which could be of great importance in the therapeutic treatments. The preliminary phytochemical results of selected solvent extracts of *E. variegata* were showed in the table 1. Phytochemical screening of aqueous leaf extract of *E. variegata* contained Glycosides, phenols and tannins. Methanolic extract revealed the presence of flavonoids, glycosides, phenols, tannins and resins. The ethylacetate extract showed the presence of alkaloids, flavonoids, phenols, tannins and resins.

The qualitative phytochemical analysis indicates that hexane extract possess flavonoids, glycosides and saponins. Dichloromethane extract were found to contain flavonoids, glycosides, phenols and tannins. From the phytochemical analysis it was noted that all the extracts of *E. variegata* leaf are rich in various secondary metabolites such as phenolics, alkaloids, flavonoids, tannins and saponins. Among the various solvents screened for phytochemicals, hexane, chloroform, dichloromethane, ethyl acetate, methanol and aqueous are very effective. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook *et al.*, 1996). Flavonoids are most commonly known for their antioxidant activity. They are modifiers which modify the body's reactions to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Balch, 2000).

The presence of alkaloids explains its anti-bacterial activity, since this phytochemical is reported to have anti-bacterial activity (Idowu *et al.*, 2003). Tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial

and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins.

Antibacterial activity of *Erythrina variegata*

The antibacterial activity has been screened because of its great medicinal relevance with the recent years, infections have increased to

a great extent and resistance against antibiotics, become an ever increasing therapeutic problem (Ali *et al.*, 2001). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial compounds. Some of the photo of experimental finding is given hereunder.

Table.1 Preliminary phytochemical screening of selected solvent leaf extracts of *E. variegata*

S.No.	Phyto-chemicals	n-Hexane	Chloroform	dichloromethane	ethylacetate	methanol	aqueous
1.	Alkaloids	-	-	-	+	-	-
2.	Flavonoids	+	+	+	+	+	-
3.	Glycosides	+	+	+	-	+	+
4.	Steroids	-	-	-	-	-	-
5.	Phenols	-	+	+	+	+	+
6.	Tannins	-	+	+	+	+	+
7.	Saponins	+	-	-	-	-	-
8.	Resins	-	-	-	+	+	-

+ = Presence, - = Absence.

Table.2 Antibacterial activity of selected solvent extracts of *Erythrina variegata* (10mg/ml)

S.No.	Bacterial strain	P + control	N - control	N-hex.	Chlof.	DCM	Ethyl acetate	Methanol	Aqueous
1.	<i>E.coli</i>	30mm	7mm	12mm	11mm	8mm	13mm	10mm	20mm
2.	<i>Pseudomonas aeruginosa</i>	30mm	7mm	8mm	11mm	8mm	8mm	10mm	20mm
3.	<i>Proteus vulgaris</i>	30mm	7mm	8mm	12mm	8mm	10mm	10mm	8mm
4.	<i>Staphylococcus aureus</i>	30mm	7mm	12mm	10mm	11mm	13mm	11mm	8mm
5.	<i>Klebsiella pneumoniae</i>	28mm	7mm	13mm	11mm	10mm	8mm	11mm	9mm

Note: P + positive control= Amikacin (10µl) and N – negative control = Sterilized Distilled water

Fig.1 Antibacterial activity of *Erythrina variegata*

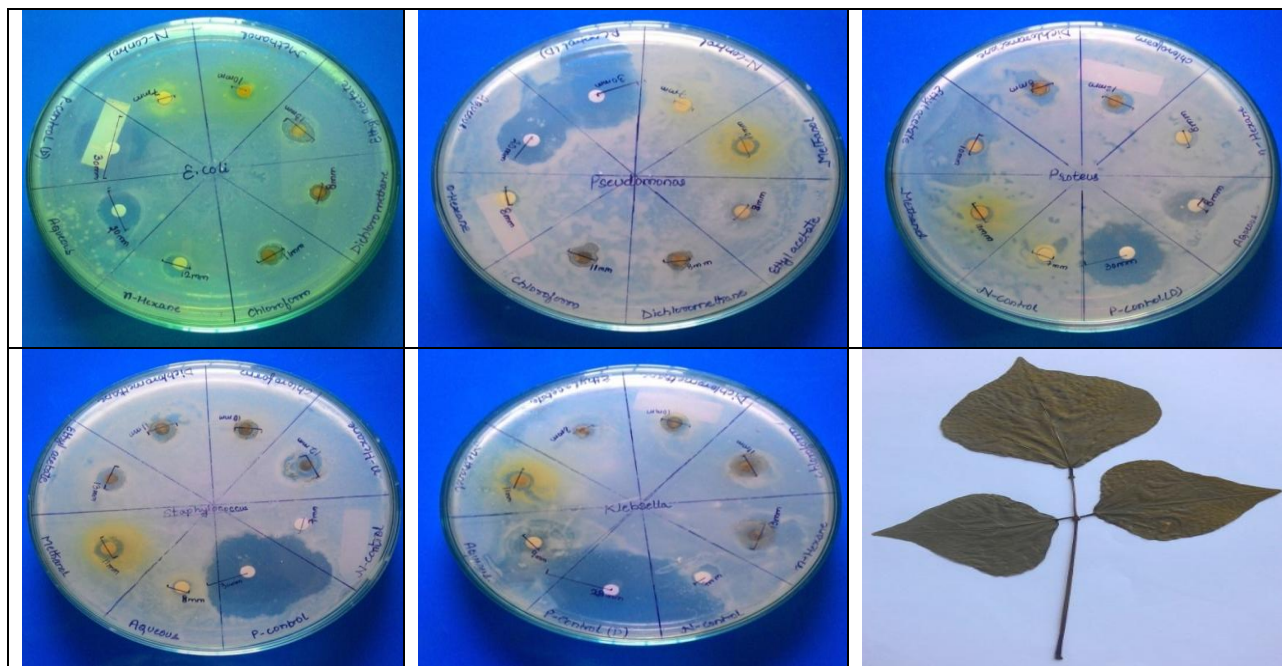


Table.2 represents the antibacterial effect of selected solvent extracts of *Erythrina variegata* by agar- disc diffusion method against selected bacterial strains and the zone of inhibition was assessed in millimeter diameter. The aqueous extract of plant showed best zone of inhibition against *Escherichia coli* and *Pseudomonas aeruginosa*. Methanolic extract showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa* and same zone of inhibition result against *Proteus*, *Staphylococcus* and *Klebsiella* (Haque *et al.*, 2006). The ethylacetate and dichloromethane showed moderate zone of inhibition against bacteria. The antibacterial activity of n-hexane of *Erythrina variegata* showed maximum zone of inhibition against *Klebsiella* comparison to others. The chloroform showed constantly best zone of inhibition in all bacteria among all other solvents. The standard antibiotic “Amikacin” showed good antibacterial activity and it is considered as standard antibacterial drugs as used today which is taken as positive control against others bacterial strains (Fig. 1).

It is concluded that the antibacterial activity of crude extract from the leaves of *E. variegata*

may be due to the presence of various phytochemical constituents indulged in them. Therefore the extracts of *E. variegata* could be recommended as a source of pharmaceutical and traditional drug materials required for preparation of antibacterial agents. This plant different solvents extract recommended further as best antibacterial drug. This study related to zone of inhibition of *E. variegata* extract seems that all the selected extract possess significant antibacterial activity against Gram positive and Gram – negative bacterial pathogens and this results seems to be interesting and notable. This may be due to the solvent extract containing different constituents having antibacterial activity.

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